



# UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

Address: COMMISSIONER FOR PATENTS

P.O. Box 1450

Alexandria, Virginia 22313-1450

www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/620,433	07/17/2003	John W. Ludlow	069952-0201	1097
22428 7590 04/14/2008 FOLEY AND LARDNER LLP SUITE 500 3000 K STREET NW WASHINGTON, DC 20007				
EXAMINER SINGH, ANOOP KUMAR				
ART UNIT		PAPER NUMBER		
1632				
MAIL DATE		DELIVERY MODE		
04/14/2008		PAPER		

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

### Office Action Summary

**Application No.**

10/620,433

**Applicant(s)**

LUDLOW ET AL.

**Examiner**

Anoop Singh

**Art Unit**

1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --  
**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 17 January 2008.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 1-3, 5-21, 26-28, 88-91, 93-96 and 98-100 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-3, 5-21, 26-28, 88-91, 93-96 and 98-100 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO/SB/08)  
Paper No(s)/Mail Date 1/17/2008
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date \_\_\_\_\_
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_

### **DETAILED ACTION**

Applicant's amendment to the claims and arguments filed January 17, 2008 have been received and entered. Claims 1, 5, 11, 13, 15, 27, 93-94 have been amended, while claims 4, 29-87, 92 and 97 have been canceled.

Claims 1-3, 5-21, 26-28, 88-91, 93-96 and 98-100 are pending in the instant application.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 01/17/2007 has been entered.

### ***Election/Restrictions***

Applicant's election with traverse of group I, claims 1-28, in the reply filed on April 14, 2006 was acknowledged. The traversal was on the ground(s) that the office has not demonstrated that it would be a burden to examine claims 1-87 together. This was not found persuasive because the office has demonstrated that the various claims fall into patentably distinct groups as supported by their different classifications. Further, each of the groups would require a unique search and require a different consideration of the relevant art within the scope of the invention as stated in previous office action dated 7/13/2006.

Claims 1-3, 5-21, 26-28, 88-91, 93-96 and 98-100 are under consideration.

***Information Disclosure Statement***

Applicants' IDS, filed 1/17/2008 and 4/2/2008 have been considered.

***Claim Objections***

Claim 93 is objected to because it depends on a cancelled claim 97.  
Appropriate correction is required.

***Withdrawn-Claim Rejections - 35 USC § 102***

Claims 1-7, 9-13, 15-21, 26, 27 provisionally rejected under 35 U.S.C. 102(e) as being anticipated by copending Application No. 09/764,359 (US patent application no: 2002/0039786; art of record) is withdrawn in view of amendments to the claim now limiting the method that uses iodixanol.

Claims 1-2, 6 rejected under 35 U.S.C. 102(b) as being anticipated by Tateno et al (EP 682106, dated 11/15/1995) is withdrawn in view of amendments to the claim now requiring iodixanol for the isolation of hepatic cells from liver of adult mammal.

***Withdrawn- Claim Rejections - 35 USC § 103***

Claims 1-10, 12-17, 26 rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Singh et al Acta Physiol Scand 117(4): 497-505, April 1983, art of record) and Naughton et al (US Patent no. 5785964, dated 7/28/1998) is withdrawn in view of amendments to the claims now reciting iodixanol for separating the hepatic cells. Applicants' arguments are moot in view of withdrawal of rejection. However, upon further consideration new rejections are presented below in view of newly submitted references.

Claims 1-6, 8, 11-17, 22-28, 88-100 rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc

Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9) and Graham (Scientific World J 2:1347-50, May 2002) is withdrawn.

Claims 1-17, 22-28, 88-100 rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9), Graham (Scientific World J 2:1347-50, May 2002) and Naughton et al (US Patent no. 5785964, dated 7/28/1998) is withdrawn in view of amendments. However, upon further consideration a new rejection is presented below in view of new art.

Claims 1-28, 88-100 rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9), Graham (Scientific World J 2:1347-50, May 2002) and further in view of Dementrious et al (US patent no. 6,140,123, dated 10/31/2000, effective filing date 10/7/1998) is withdrawn in view of arguments and amendments to the claims. However, upon further consideration a new rejection is presented below in view of newly submitted references.

### ***New Grounds of Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

1. Claims 1-3, 5, 11-17, 27, 88, 90-91, 94-95, 98-99 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasui et al (Hepatology, 1997, 25: 329-334, IDS),

Tateno et al (EP 682106, dated 11/15/1995, art of record), Graham (Scientific World J 2:1347-50, May 2002, art of record)/ Graziani-Bowering et al (Journal of Immunological Methods 207 (1997) 157-168, IDS) and Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9, art of record).

With respect to claim 1, 27 and 94 Yasui et al teach isolation of oval cells from liver by obtaining a liver comprising hepatic cells , perfusing the liver with a chelation buffer, digesting a rat liver using two step collagenase digestion and dissociating the digested liver, and filtering to then obtain cell suspension. Yausi et al teach gently loading the cell suspension onto a discontinuous percoll gradient and then centrifuging the gradient to obtain cells at the bottom fraction of the tube to obtain a population of oval cell (see page 329, col. 2, see material method) to further characterize the cell population by using surface marker and diameter of the cell. It is noted that method of isolating oval cells that are known to be precursor of hepatic cells disclosed by Yausi have a diameter of 10-15  $\mu\text{M}$  and shows characteristics of hepatocyte and biliary epithelial cell and express markers cytokeratin 18 and 19 (see page 332, col. 1, para. 1 bridging to col. 2). Additionally, these cells upon transplantation produced mature hepatocyte (see page 333, col. 1, para. 1) and produced albumin (see figure 8). Although, Yasui et al teach a method to obtain and enrich similar population of viable hepatic cell as those specifically embraced by instant claims but differed from claimed invention by not teaching suspending the suspension of cells in medium comprising iodixanol or collecting a band having density less than 1.0792.

Tateno et al teach a method to obtain liver parenchymal cell having clonal growth ability that are considered to contain hepatic progenitor cells (see abstract and page 3, line 4-5). It is noted that method disclosed by Tateno et al embrace isolating hepatic cells from liver of adult mammal by the collagenase perfusion and percoll centrifugation (see table 1, page 6-7). Additionally, Tateno et al embraced the potential of hepatocytes of human and all other mammals having clonal

growth ability from various animal species (see page 5, lines 51-55). Although, Tateno et al teach a method to isolated hepatic cell including progenitor cells and generally embraced the idea that liver parenchymal cells have clonal growth and contains hepatic progenitor cells, which could be selected or enriched with specific markers for therapeutic purposes (see page 5). However, Tateno et al differed from claimed invention by not using Optiprep (iodixanol) for isolation and enrichment of smaller hepatocytes and cells of 7-12 microns. Prior to instant invention, strategy of using a density barrier including use of iodixanol to isolate a low-density cell fraction is to layer the crude cell suspension over a barrier whose density is just above that of the cell of interest was known to one of ordinary skill in the art. For instance, although the density barrier for hepatic stellate cells is less than  $<1.07$  g/ml, however, in order to improve their purity the density gradient could be adjusted. In this regard, Graham et al teach that majority of parenchymal cells from mammalian liver cells are routinely prepared by collagenase and pronase digestion of the liver using a tissue perfusion system that is separated by very low speed centrifugation (50 g) (see abstract). It is noted that Graham et al disclose using a simple low-density barrier (1.096 g/ml) that is required to remove the remaining parenchymal cells from the supernatant which contains all of the lower density nonparenchymal cells. Graham et al disclose flotation through a low-density iodixanol barrier could provide a satisfactory enrichment of the least dense nonparenchymal cell and the stellate cells. Specifically, Graham disclose different strategies to isolate cells wherein concentration of iodinated density gradient medium is equivalent to approx 1.067 g/ml with iodixanol or a lower-density concentration (1.053 g/ml) (See references therein such as Cassimanis) or by placing the cell suspension in the top layer ( $\rho = 1.067$  g/ml) rather than the bottom layer ( $\rho = 1.096$  g/ml) (See pages 1348, ,methods and 1349, notes) . Likewise Graziani-Bowering et al disclose the disadvantages of using sucrose and Percoll gradient-density media to isolate and enrich cells (see references of Ford et al., 1994 cited

therein). Graziani-Bowering et al specifically describe percoll gradient-density medium that has lower osmolality and viscosity than sucrose gradient however, it requires a very high g-forces to generate the gradients to isolate cells (see page 158, col. 2, para. 2). Although, Graziani-Bowering enriched monocyte but provided adequate guidance with respect to several approaches to increase the yield. These approaches included diluting optiprep to decrease cell load, increasing centrifugation time to give cells longer to float to the 1.068 g/ml layer reducing volume to decrease cell load, reducing the volume of the 1.078 g/ml layer from 10 to 7.5 ml to decrease the distance through with the cells had to float to reach the 1.068 g/ml layer, and changing the density of the solution from 1.078 to 1.074 g/ml to reduce the density difference between the two different cell density specific layers, thus reducing the concentration of cells at the interface of these layers (see Figure 1 and page 166, col. 1, para. 2). However, Graham/ Graziani-Bowering et al differed from claimed invention by not disclosing isolation of hepatic cell that included progenitor cells.

The deficiency of Yasui and Graham/ Graziani-Bowering is cured by Brill et al who teach a method for identifying and isolating antigenically related cell populations present in normal tissues using monoclonal antibodies to oval cell antigens and fluorescence-activated cell sorting. It is noted that Brill et al disclose three cellular subpopulations that could be isolated including (i) committed progenitors to hepatocytes; (ii) committed progenitors to bile ducts; or (iii) a mixed population of hemopoietic cells that contained a small percentage of hepatic blasts that are possibly pluripotent. Brill et al also teach that the hepatic blasts are small (7-10 microns) cells that differentiate into cells with recognizable parenchymal cell fates (see abstract). It is noted that although Brill et al provided adequate guidance of presence of distinct population of hepatic cell including hepatic progenitor, he did not teach method to isolate small size cell using gradient centrifugation method for isolation of cells.



Accordingly, in view of the teachings of Yasui et al, Tateno et al, Graham/ Graziani-Bowering and Brill, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to improve the method of obtaining hepatic cells by replacing the percoll based cell separation medium with a iodixanol (optiprep) barrier based density gradient with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Brill had already disclosed the presence of mixed cellular subpopulations that could be isolated separately including committed progenitors to hepatocytes; committed progenitors to bile ducts; or a mixed population of hematopoietic cells. It would have been *prima facie* obvious to one of ordinary skill in the art to use flotation of cells through a low-density iodixanol barrier to provide satisfactory enrichment of hepatic cells as taught by Graham/ Graziani-Bowering, particularly since the hepatic blasts are only 10 microns (*supra*) and both Yasui and Brill et al sought to isolate hepatic cells including stem and progenitor cells. Given that method to isolate hepatic cells that expressed marker of hepatic and biliary epithelia having diameter of 10-15  $\mu\text{M}$  using percoll method were known in prior art, it would have been *prima facie* obvious and routine optimization for one of the ordinary skill to use other functionally equivalent gradient system such as low-density iodixanol barrier taught by Graham/ Graziani-Bowering. It is noted that Graham/ Graziani-Bowering provided adequate guidance to optimize the concentration of iodixanol and the density of resulting interface band by characterizing the cells obtained at the interface expression of the cell surface markers and by determining the diameter of the cells as disclosed by Yasui and Brill.

One who would practice the invention would have had reasonable expectation of success because Yasui and Tateno et al had already described the method to isolate mammalian hepatic cells using density gradient centrifugation method. Additionally, Yasui and Brill, had already described the presence of low density

nonparenchymal cell, while Graham/ Graziani-Bowering et al suggested that low density low density iodixanol based gradient method could be used to isolate and enrich cells. Thus, it would have only required routine experimentation to modify the method disclosed by Yausi to substitute percoll gradient with another functionally equivalent iodixanol (optiprep) based gradient to isolate hepatic cells including progenitor cells as disclosed by instant invention.

The limitation of claims 13-17 and 90-91 and 98-99 are included in the instant rejection since this buffer comprising RPMI-1640 medium with 10% human or bovine serum, or filtering step, centrifugation speed and machine, collection bags as required by the claims are obvious variations of the medium, filtration speed, machine and collection bags disclosed by cited arts. It is emphasized that in absence of any unexpected result one of ordinary skill would have been sufficiently aware of the different analogous medium in presence or absence of phenol red or centrifugation machine and relative g force depending upon the rotor radius.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

2. Claims 26 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasui et al (Hepatology, 1997, 25: 329-334, IDS), Tateno et al (EP 682106, dated 11/15/1995, art of record), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9) and Graham (Scientific World J 2:1347-50, May 2002) / Graziani-Bowering et al (Journal of Immunological Methods 207 (1997) 157-168, IDS) as applied to claims 1-3, 5, 11-17, 27, 88, 90-91, 94-95, 98-99 above, and further in view of de Boer et al (Journal of Pathology, 1999, 188: 201-206) and Gordon et al (Blood, 2001, Vol. 98, 11, page 657a).

The combined teaching Yausi, Tateno et al, Brill and Graham/ Graziani-Bowering have been discussed above and relied in same manner here. However,

none of the references teaches EP-CAM or CD133 marker for hepatic progenitor cells.

However, prior to instant invention, de Boer et al teach that the majority of hepatocytes express Ep-CAM in an 8-week embryonic liver. de Boer et al also teach that during precursor cell differentiation into mature hepatocytes, several intermediate morphological stages also show expression of Ep-CAM. It is noted that only full maturation of the precursor resulted in the disappearance of Ep-CAM expression. The results of de Boer et al suggest that expression of Ep-CAM is a prerequisite of the proliferative phenotype during differentiation of hepatocyte precursors (see abstract). Gordon et al provided guidance with respect to CD133 (formerly AC133) is a novel 5-transmembrane glycoprotein cell surface antigen selectively expressed on CD34bright stem cells in human bone marrow, fetal liver that contain more primitive pluripotent stem cells including CD34 negative stem cell (abstract). However, de Boer et al and Gordon both differed from claimed invention by not disclosing method to isolated hepatic cell including stem cell.

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to modify the method of obtaining hepatic cells enriched in viable hepatic cells taught by Yausi, Tateno, Brill and Graham/ Graziani-Bowering et al by further characterizing the cells at the interface by sorting cells that have diameter of approximately 10-15  $\mu\text{M}$  and are also positive for Ep-CAM, CD133 or both using cell sorting method with reasonable expectation of achieving predictable result particularly since de Boer et al and Gordon both suggested that expression of Ep-CAM is a prerequisite of the proliferative phenotype during differentiation of hepatocyte precursors, while CD133 is expressed in fetal liver which is marker for more primitive pluripotent stem cells. Thus, it would have only required routine experimentation to modify the method disclosed by Yausi to substitute percoll gradient with another iodixanol (optiprep) based gradient taught by Graham/

Graziani-Bowering to isolate hepatic cells at the interface band by characterizing using the markers disclosed by Brill, DeBoer and Gordan.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

3. Claims 6-8, 89 and 96 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasui et al (Hepatology, 1997, 25: 329-334, IDS), Tateno et al (EP 682106, dated 11/15/1995, art of record), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9) and Graham (Scientific World J 2:1347-50, May 2002)/Graziani-Bowering et al (Journal of Immunological Methods 207 (1997) 157-168, IDS) as applied to claims 1-3, 5, 11-17, 27, 88, 90-91, 94-95, 98-99 above, and further in view of Naughton et al (US Patent no. 5785964, dated 7/28/1998, art of record).

The combined teaching Yausi, Tateno, Brill and Graham/ Graziani-Bowering have been discussed above and relied in same manner here. However, none of the references teaches mechanical dissociation or use of different protease such as elastase, collagenase and neutral protease.

Prior to filing of this application, Naughton et al teach that cells have been routinely harvested from tissue or organ by mechanically dissociation and/or treatment with digestive enzymes and/or chelating agents to weaken the connections between neighboring cells enabling to disperse the tissue into a suspension of individual cells. Naughton et al also disclose that enzymatic dissociation could be accomplished by mincing the tissue and treating the minced tissue with any of a number of digestive enzymes including collagenase and elastase, pronase and/or neutral protease such as dispase, (see col. 9, lines 20-25)

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to modify the method of obtaining cells enriched in viable

hepatic cells taught by Yausi, Tateno, Brill and Graham/ Graziani-Bowering by using other method to dissociate cells from the liver as disclosed by Naughton et al.

One of ordinary skill in the art would have been motivated to mince or mechanically dissociate or use other protease to obtain population of hepatic cell particularly since Naughton et al had already disclosed that these could be used in conjunction with collagenase, dispase and/or elastase in order to obtain cells derived from liver.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

4. Claims 18-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasui et al (Hepatology, 1997, 25: 329-334, IDS), Tateno et al (EP 682106, dated 11/15/1995, art of record), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9) and Graham (Scientific World J 2:1347-50, May 2002)/ Graziani-Bowering et al (Journal of Immunological Methods 207 (1997) 157-168, IDS) as applied to claims 1-3, 5, 11-17, 27, 88, 90-91, 94-95, 98-99 above, and further in view of and further in view of Dementriou et al (US patent no. 6,140,123, dated 10/ 31/ 2000, effective filing date 10/7/1998, art of record).

The combined teaching Yausi, Tateno, Brill et al and Graham/ Graziani-Bowering have been discussed above and relied in same manner here. However, none of the references teaches that the cells that are subjected to cryopreservation.

Prior to filing of this application, Demetriou et al teach that cells have been routinely harvested and preserved in scientific research and development. It is also noted that Demetriou et al teach that cell could be re-used after thawing and placing in a cell culture medium. Demetriou et al also disclose storage medium for cryopreservation (col. 1-2) including cryopreservation buffer comprising serum and

DMSO (See entire col. 6 and 7). However, Demetriou et al do not specifically teach cryopreservation of hepatic cells.

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to subject the method of cultured cells taught by Yausi, Tateno, Brill et al, Graham/ Graziani-Bowering et al to cryopreserve as taught by Demetriou et al.

One of ordinary skill in the art would have been motivated to cryopreserve expanded hepatic cells including progenitor cells for future analysis or use as described by Demetriou et al.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

5. Claim 100 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yasui et al (Hepatology, 1997, 25: 329-334, IDS), Tateno et al (EP 682106, dated 11/15/1995, art of record), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9, art of record) and Graham (Scientific World J 2:1347-50, May 2002, art of record)/Graziani-Bowering et al (Journal of Immunological Methods 207 (1997) 157-168, IDS) as applied to claims 1-3, 5, 11-17, 27, 88, 90-91, 94-95, 98-99 above, and further in view of and further in view of McMannis (US patent 531,6540, dated 5/31/1994) .

The combined teaching Yausi, Tateno, Brill et al and Graham/ Graziani-Bowering have been discussed above and relied in same manner here. However, none of the references teach centrifugation is performed in COBE 2991 cell processor.

McMannis et al describe that centrifugation utilizes the principle that constituents (e.g., cells, cell clusters) within a medium (e.g., liquid solutions/mixtures) that assume a particular radial position within the centrifuge bowl based upon their respective densities and will therefore separate when the

centrifuge is rotated at an appropriate angular velocity for an appropriate period of time. McMannis disclose that COBE 2991 cell processor was commercially available which provided centrifugation system that enhanced the potential for maintaining a desired degree of sterility in various of the aspects involved in/relating to the actual separation of such constituents from the medium (See col. 1, lines 15-35).

Additionally, McMannis et al also teach that instead of having the discontinuous density gradient within a tube, the gradient could be provided to flexible processing bag for sequential removal of fractions to obtain the desired cells at one or more of the interfaces (see col. 3, lines 22-33).

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to improve the method of centrifugation taught by Yausi, Tateno, Brill et al and Graham/ Graziani-Bowering by substituting centrifuge tube and conventional centrifuge with another functionally equivalent bag and COBE 2991 cell processor as taught by McMannis with reasonable expectation of achieving predictable result particularly since McMannis provided advantages of using bag and COBE 2991 system in separating cells using gradient fractionation. Thus, it would have only required routine experimentation to modify the method disclosed by Yausi and Graham/ Graziani-Bowering to substitute tube and centrifuge with another functionally equivalent bag and cell processor taught by McMannis to isolate hepatic cells at the interface band by characterizing using the markers disclosed by Brill.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

***Withdrawn-Double Patenting***

Claims 1-28 remain provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 4, 6-9, 12-21, 23-34 of copending Application No. 09/764,359 (published as 2002/0039786 A1) is withdrawn in view of amendments to the independent claim 1 now requiring specific limitation that were not taught by prior application.

***Conclusion***

No Claims allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Ford et al (Analytical Biochemistry, 1994, 220, 360-366, IDS).

Claasems et al (Human Reproduction , 13, 1998, 3139-314, IDS).

Kubota et al ( Proc. Nat. Acad. Science, 2000, 97, 22, 12132-12137).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.



Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Anoop Singh  
AU 1632

/Thaian N. Ton/  
Primary Examiner, Art Unit 1632